79. (Additional) The polypeptide according to claim 78, wherein the substitutions consist of alanine for residue 45, glycine for residue 102, arginine for residue 189, and valine for residue 195.

REMARKS

Claims 35-64 are pending in the application. Claims 43-51 have been allowed; claims 35-38, 40, 41 and 52-64 have been rejected; and claims 39 and 42 have been objected to.

No new matter is introduced by the amended and additional claims.

Support for amended claim 35 is found at page 32, lines 20-32, Table 5A, page 51, and page 47, line 6, of the application as originally filed. Furthermore, claim 35 has been amended in a manner that meets the Examiner's conditions of Enablement:

> [B]ecause the specification, while enabling for a refolded, modified, hemolytically attenuated, penumolysin polypeptide obtained by mutating the nucleic acid molecule encoding type 14 wild type pneumolysin having SEQ ID NO:3 in a region comprising 17 and 18, 33, 41 through 46, 61 through 66, 83, 101, 102, 127, 128, 148, 172, 189, 195, 255 and 257...(page 6, section 10, of the April 10, 2001, office action).

Amended claim 35 pertains to pneumolysin polypeptides comprising SEQ ID NO:3 that are modified, partially-soluble and attenuated in hemolytic activity, and wherein the modification comprises at least one amino acid substitution selected from the group consisting of residues 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257. The addition of the element of 'partially-soluble' is in replacement of the element of refoldability, although the instant application states that partial solubility is a probabilistic indicator of refoldability (page 48, lines 6-8). Thus, applicants contend claim 35 does not introduce new matter and that claim 35 has been amended within the Examiner's bounds of Enablement.

Claims 53, 60, and 62 have been amended by changing their dependency from

cancelled claim 52 to claim 35. Claim 64 has been amended by deleting the term 'derived'.

Support for additional claims 65-67 is found at page 23, Table 1 and lines 10-17, of the application as originally filed. Support for additional claim 68 is found at page 52, Table 5B. Support for additional claims 69-74 and 78-79 is found at page 51, Table 5A. Support for additional claims 75-77 is found at page 51, Table 5A, and at page 24, Table 2 and lines 5-11.

Applicants further request a change of the docket number of the instant application. Accordingly, it is requested that Docket No. "1758-4036US2" be deleted and replaced with -- 3842-4036US2 --.

RESPONSE TO § 112, SECOND PARAGRAPH, REJECTION

Claims 38-41 and 56-59 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim subject matter. In particular, claims 38-41 and 56-59 were rejected due to the internal inconsistency of the terms 'residue' and 'position'. Applicants have cancelled claims 38-41 and 56-59, and thus the rejection is moot.

Claim 64 stands rejected under 35 U.S.C. § 112, second paragraph, as being indefinite due to the use of the term 'derived.' The term has been deleted from the claim. Applicants respectfully request withdrawal of this ground of rejection.

RESPONSE TO § 112, FIRST PARAGRAPH, REJECTION

Claims 38 and 56 stand rejected under 35 U.S.C. § 112, first paragraph, because the Examiner contends that the specification does not provide enablement for a refolded, modified pneumolysin mutant having amino acid substitutions at more than one position selected

from the group consisting of position 61, 148 and 195. Although applicants have cancelled claims 38 and 56, applicants respectfully disagree with this ground of rejection. Applicants respectfully submit additional claims 65-67, which encompass modified pneumolysin polypeptide mutants having amino acid substitutions at more than one position selected from the group consisting of positions 61, 148 and 195. Furthermore, applicants have amended these and the remaining claims by deleting the element of refoldability.

Applicants contend that the specification does enable a modified pneumolysin mutant that is partially-soluble, with reduced hemolytic activity, having amino acid substitutions at more than one position selected from the group consisting of position 61, 148 and 195. As a substitution at any one of these amino acids resulted in reduced hemolytic activity (page 23, lines 4-6, and page 53, table 6), one skilled in the art would reasonably expect that a pneumolysin mutant, having one (or more) of these same substitutions and additional substitutions, would also possess reduced hemolytic activity. In fact, it would be rather surprising if a substitution of a particular residue(s) in combination with 61, 148, and/or 195, resulted in the return of hemolytic activity.

In addition, applicants contend that the specification enables one skilled in the art to determine without undue experimentation whether a partially-soluble pneumolysin polypeptide having a substitution at residues 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, in addition to one or more substitutions at residues 1-257, attenuates hemolytic activity. The Examiner contends that "The art reflects that refoldability of a protein and attenuation of the hemolytic activity of a wild-type pneumolysin by random mutations are unpredictable events." (page 4, September 25, 2001 Official Action). As applicants have deleted the element of refoldability in the claims, the question now pertains to

whether the disclosure requires undue experimentation to identify partially-soluble pneumolysin mutants having at least one specific substitution as detailed above, in addition to one or more mutations and reduced hemolytic activity.

Applicants contend that in considering the factors for determining whether a disclosure would require undue experimentation, the instant application does not require undue experimentation as claimed. The factors considered to determine undue experimentation are: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. (*In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Circ. 1988)).

Applicants contend the quantity of experimentation is not undue because the experimentation is routine and the specification provides a reasonable amount of guidance as to how to obtain partially-soluble pneumolysin mutants and how to identify whether pneumolysin mutants possess attenuated hemolytic activity.

> The test [for undue experimentation] is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the claimed invention. (*PPG Indus., Inc. v. Guardian Indus. Corp.*, 75 F.3d 1558, 1564, 37 USPQ 2D 1618, 1623 (Fed. Cir. 1996)).

The specification provides guidance for obtaining pneumolysin mutants, as described in Example 4, "Random Mutagenesis to Generate Modified Pneumolysin", on pages 45-46. This protocol revolves around PCR, which in the current state of the art, is routine experimentation. The

guidance to determine whether modified pneumolysins are partially-soluble is described in Example 5, section (c), "testing for expression of modified pneumolysin polypeptides in the soluble fractions", on page 48. The test for solubility only entails growing expression vectors containing modified pneumolysins in bacteria, inducing expression, lysing the cells and testing the soluble fractions for hemolytic activity (see below). Applicants contend that the expression of recombinant protein in bacteria is routine in the art. Also, the specification provides guidance to assess the hemolytic activity of modified pneumolysins, as described in the subsection, "hemolysis inhibition assay by modified pneumolysin", of Example 11 on page 70. This screening method only entails the incubation of erythrocytes with the mutant pneumolysins and the observation of whether the erythrocytes lyse. In respect to the state of the art, this experiment is routine. Although the Examiner states: "There appears to be no evidence within the instant specification enabling a double or triple pneumolysin mutant comprising two or three amino acid substitutions at positions 61, 148 and 195 of SEQ ID NO:3, which mutant is refolded and has attenuated hemolytic properties." (page 4 of Official Action dated September 25, 2001), none of the factors in determining undue experimentation require "evidence". Rather, the factors which may be considered for enablement require a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to obtain partially-soluble pneumolysin mutants and to identify hemolytic attenuation.

Applicants further contend the specification provides working examples of how to obtain poly-substituted, partially-soluble pneumolysin mutants and to identify whether these mutants are hemolytically attenuated. In Table 5A (page 51), the application presents five multiply-substituted mutants that possess reduced hemolytic activity (Table 4, page 50). Although the specification discloses that single point mutations are preferred because the

antigenic nature of the native pneumolysin is more likely to be preserved, the specification does state "a combination of multiple mutations, may be used." (page 25). Furthermore, the Examiner refers to the instant specification as stating that multiple mutations are unpredictable. However, the specification states that "multiple mutations are sometimes unpredicatable", and even in the rare event that the multiple mutations "may act synergistically to abolish activity", the application provides guidance to routine experimentation to determine whether hemolytic activity is attenuated.

In sum, the specification enables the claimed invention, namely that the specification provides an appropriate level of guidance to one skilled in the art to determine whether modified partially-soluble pneumolysins with at least one mutation at residues 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257, possess reduced hemolytic activity.

The Examiner states that "There is no certainty that the resultant modified pneumolysin would retain the functional integrity or biological/immunogenic competence of the native pneumolysin, without rendering it non-functional" (page 6, September 25, 2001 Official Action, emphasis added). The Examiner's requirement of "certainty" to satisfy enablement misapplies the law as certainty is not a condition in determining enablement.

In re Vaeck, 947 F.2d 488, 496, 20 USPQ 2D 1438 (Fed. Circ. 1991), the Federal Circuit held that enablement of a generic claim could be satisfied in an "unpredictable art", i.e. an art where "certainity" would not exist.

> In so doing we do *not* imply that patent applicants in art areas currently denominated as "unpredictable" must never be allowed generic claims encompassing more than the particular species disclosed in their specification. It is well settled that patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art. *In re Angstadt, 537*

> *F.2d 498, 502-03, 190 U.S.P.Q. (BNA) 214, 218 (CCPA 1976).* However, there must be sufficient disclosure, either through illustrative examples or terminology, to teach those of ordinary skill how to make and how to use the invention as broadly at it is claimed. (Emphasis added).

Applicants' specification provides ample direction to obtain pneumolysin mutants within the full scope of the claims and to determine whether they are hemolytically attenuated. The guidance to obtain and identify claimed pneumolysins is not beyond the state of the art or the relative skill of those in the art, as the mutagenesis methods may be PCR-based, the solubility test may be based on recombinant protein expression and the hemolysis screen simply involves incubation and routine detection. Lastly, the breadth of applicants' claims is not overly-broad since the modified pneumolysins must comprise at least one specific mutation as described in claim 35. Therefore, applicants respectfully request reconsideration and removal of this ground of rejection.

Claims 40, 41, 58, and 59 stand rejected under 35 U.S.C. § 112, first paragraph, because the Examiner contends that the specification does not provide enablement for a modified, refolded, attenuated pneumolysin mutant having a substitution of hydroxyproline at position 61, arginine or histidine at position 148, and leucine, glycine or alanine at position 195. Although claims 40, 41, 58 and 59 have been cancelled, applicants respectfully disagree with this ground of rejection.

As discussed above, applicants' specification enables a modified, attenuated pneumolysin mutant having a substitution of proline or hydroxyproline at position 61, arginine or histidine at position 148, and leucine, glycine or alanine at position 195. The specification provides working examples of amino acid substitutions at these positions that cause the attenuation of hemolytic activity (page 51, Table 5A). As the specification shows a substitution

of proline at position 61, lysine at position 148, or isoleucine/valine at position 195 causes attenuated activity, it is reasonable to predict that similar substitutions (in terms of amino acid charge) of hydroxyproline at 61, arginine or histidine at 148, and leucine, glycine or alanine at 195 would also cause an attenuation of hemolytic activity. Furthermore, as stated previously, the specification provides guidance as to how to determine whether specific amino acid substitutions would render pneumolysins hemolytically-attenuated. Thus, additional claims 65-68 are enabled by the specification and do not require undue experimentation. Applicants respectfully request withdrawal of this ground of rejection.

RESPONSE TO § 102 REJECTION

Claim 35 has been rejected under 35 U.S.C. § 102(b) as being anticipated by Lock et al. Specifically, the Examiner argues that Lock et al. show a modified pneumolysin mutant having a substitution at position 172. Applicants have amended claim 35 to address the Examiner's concerns. Specifically, the claimed modified pneumolysins comprise at least one mutation selected from a group consisting of residues which do not list position 172. Applicants respectfully request withdrawal of this ground of rejection.

Claims 35-37, 52-55 and 62 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Hill et al. Specifically, the Examiner refers to modified pneumolysins having a single mutation at positions 31, 127, or 156. Applicants have amended independent claim 35 to address the Examiner's concerns. Specifically, the claimed modified pneumolysins comprise at least one mutation selected from a group consisting of residues which do not list positions 31, 127 or 156. Thus, the claims are not anticipated by Hill et al. Claims 52-55 have been cancelled, and claim 62 has been amended such that it is dependent upon claim 35. Applicants respectfully request withdrawal of this ground of rejection.

None of the amended and additional claims preclude polypeptides having the mutations reported in the cited references, provided at least one of the substitutions recited in the claims is present.

RESPONSE TO § 103 REJECTION

Claims 52 and 60-64 stand rejected under 35 U.S.C. § 103 as being unpatentable over Paton et al. in view of Hill et al. and Krishnamurthy et al. or Lee et al. Paton reports two modified pneumolysins with attenuated hemolytic activity, one with a substitution at position 428, the other with a substitution at position 433. Hill reports a modified, hemolytically attenuated pneumolysin having a substitution at position 172. The Examiner contends that Krishnamurthy and Lee disclose the cross-reactivity of *pneumococcal* capsular polysaccharides between serotypes and between *Klebsiella* K2.

Applicants have cancelled claim 52, and have amended the claims such that claims 60-64 are now dependent upon amended claim 35. Amended claim 35 recites that the modification of the partially-soluble pneumolysin polypeptide comprises at least one amino acid substitution selected from the group consisting of residues 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255 and 257. This group of residues does not list residue 172 (Hill), nor residues 428 or 433 (Paton). In addition, the combination of Hill, Paton, Krishnamurthy and Lee does not teach or suggest partially-soluble modified pneumolysins. The element of partial-solubility is in reference to the teaching of the instant application that "Modified pneumolysin polypeptides expressed in both the soluble fraction and inclusion bodies are more likely to be refoldable." (page 48) The advantage of having the instant application's novel method of identifying modified pneumolysins that are at least partially-soluble, and thus possibly refoldable, is to have an efficient pre-selection of candidates for refoldability testing

(i.e. soluble pneumolysins are tested for refoldability, for example, by chromatography analysis (page 49)). The significance of refoldability is taught by the instant application, namely that if modified-attenuated pneumolysins are refoldable, it is more likely that their hemolytic attenuation is due to a functional mutation and not to a structural mutation that causes the loss of native-antigenic structure. Therefore claims 60-64 are not obvious in view of the combination of Paton, Hill, and Krishnamurthy or Lee, because this combination neither teaches nor suggests modified, partially-soluble pneumolysins that comprise at least one substitution at residues 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255 and 257.

RESPONSE TO OBJECTION

Claims 39 and 42 are objected to for being dependent from the rejected base claim 35. Claim 39 has been cancelled. Claim 35 has been amended to address the Examiner's §102(b) rejections (Lock et al., Hill et al.), by claiming modified pneumolysins, having attenuated hemolytic activity, comprising at least one mutation selected from a group consisting of residues which do not list position 172 (Lock et al.), or 31, 127, or 156 (Hill et al.). Applicants respectfully request withdrawal of this objection.

AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required for this amendment, or credit any overpayment to Deposit Account No. 13-4500, Order No. 3842-4036US2. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

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Dated: March 13, 2002

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APPENDIX

35. A modified pneumolysin polypeptide [comprising SEQ ID NO: 3], wherein the polypeptide is [modified] partially soluble, [refolded, and] has attenuated hemolytic activity, and wherein the modification of the polypeptide [has] comprises an amino acid sequence having SEQ ID NO:3 modified to possess at least one amino acid substitution [in the region comprising amino acids 1 to 257] selected from the group consisting of (residues) 17, 18, 33, 41, 45, 46, 61, 63, 66, 83, 101, 102, 128, 148, 189, 195, 239, 243, 255, and 257.

53. The polypeptide according to claim [52] 35, wherein the polypeptide is obtained by randomly mutating a nucleic acid molecule encoding a pneumolysin polypeptide.

60. The polypeptide according to claim [52] 35, wherein the polypeptide is conjugated to a polysaccharide which elicits antibodies cross-reactive with a bacterial polysaccharide.

62. [A] The vaccine comprising the polypeptide according to claim [52] 35 and a pharmaceutically acceptable carrier.

64. The vaccine according to claim 63, wherein the polysaccharide is a bacterial polysaccharide and is [derived] from a bacterium selected from the group consisting of *Haemophilus influenzae* type b; meningococcus group A, B, or C; group A streptococcus or group B streptococcus type Ia, Ib, II, III, V, or VIII; and one or more of serotypes 1-23 of *S. pneumoniae*.